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Frequency of A Very Rare 35delG Mutation in Two Ethnic Groups of Iranian Populations

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Abstract

The 35delG mutation in the Connexin 26 gene (Cx26), at the DNFB1 locus is the most common mutation in the patients with autosomal recessive non-syndromic hearing loss (ARNSHL). We have studied a total of 224 deaf cases from 189 families in two populations of Iran (Sistan va Bluchestan and Hormozgan provinces) by prescreening nested PCR, polyacrylamide gel electrophoresis and consequent direct sequencing method for all cases. The aim of the present work was to find prevalence of GJB2 mutations in the populations studied. Four different GJB2 mutations including 35delG, W24X, R127H and (V27I + E114 G) were identified in 11 of 189 families (5.8%). Two polymorphisms (V27I and V153I) also were detected in 14 families. A polymorphism S86T was determined in all cases. Homozygote 35delG mutation was found only in 1 of 189 families (0.5%).The rate of Cx26 mutations found in this study was lower than other Iranian populations. So the cause of deafness in the populations studied remains to be detected in other loci or genes.

Keywords: *Connexin 26, GJB2, Deafness, Mutation, Autosomal recessive non-syndromic hearing loss, Iran*

Introduction

ARNSHL is a frequent form of genetic hearing impairment (1). Epidemiological studies have shown that, 1 in 1000 infants are born with sever to profound hearing impairment (2). Mutations in GJB2 gene encoding Connexin 26 (Cx26) protein is associated with ARNSHL cases (3). The GJB2 gene, which is expressed in the inner ear, is thought to be important in maintaining endocochlear potential (2). Gap junction proteins, facilitate intercellular communication by encoding channels that directly link the cytoplasm of adjacent cells (4). Cx26 mutations cause sever to profound hearing impairment, but serial audiograms showed no evidence of progression of the hearing impairment or differences in the severity of the hearing impairment in affected siblings (2). More than 60 different mutations in the Cx26 gene have been reported to be responsible for autosomal recessivse inheritance of deafness (5). A single mutation, 35delG in the Cx26 gene accounts for 34 -50% of Cx26 mutations and is the most common mutation in patients with ARNSHL (5).

In the present study, we have investigated the GJB2 gene mutations in 224 ARNSHL cases of

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189 deaf families from two different population from Sistan va Baluchestan and Hormozgan provinces.

Materials and Methods

The patients were deaf students and their siblings between 2 and 33 years (mean: 13.7) originating from two different parts of Iran including Sistan va Baluchestan and Hormozgan provinces. Altogether 224 presumed autosomal recessive non-syndromic deaf cases from 189 families were studied.

There was no evidence of any obvious syndrome. The pedigrees showed autosomal recessive pattern. All patients had mild to profound sensory neural hearing loss. About half of the parents (54%) were related but when parents originated from the same village or town, the family was considered possibility consanguinity as they might carry the same mutant alleles. All the patients and deaf families were informed medical histories, pedigree information and bloods were collected with their consents.

DNA were extracted from peripheral blood samples following the standard procedure. The procedures, primers and methods of analysis have been described elsewhere. In order to determine the prevalence of the 35delG common mutation, a simple and accurate method of prescreening nested PCR and subsequent polyacrylamide gel electrophoresis were used for all patients. The entire coding sequence of cx26 gene (Genebank accession#M86849) was amplified using primers CX148F2 5`CCTGTGTTGTGTGCATTCGTC3` / CX929R3 5`CTCATCCCTCTCATGCTGTC3` (782 bp) at an annealing temperature of 59 °C. The amplified product was then diluted and used as a template for a second round of PCR using primers CX2-10F4 5`CACGCTGCAGACGATCC3` / CX2-52R4 5`GGTGGAGTGTTTGTTCAC3`(43 bp) at an annealing temperature of 54°C. The amplified products were separated by electrophoresis on a 15% polyacrylamide gel and then products were visualized by silver staining (fig. 2). The 35delG mutations were detected by identification of two separated bands of 43 bp for the wild type and 42 bp for the mutant allel.

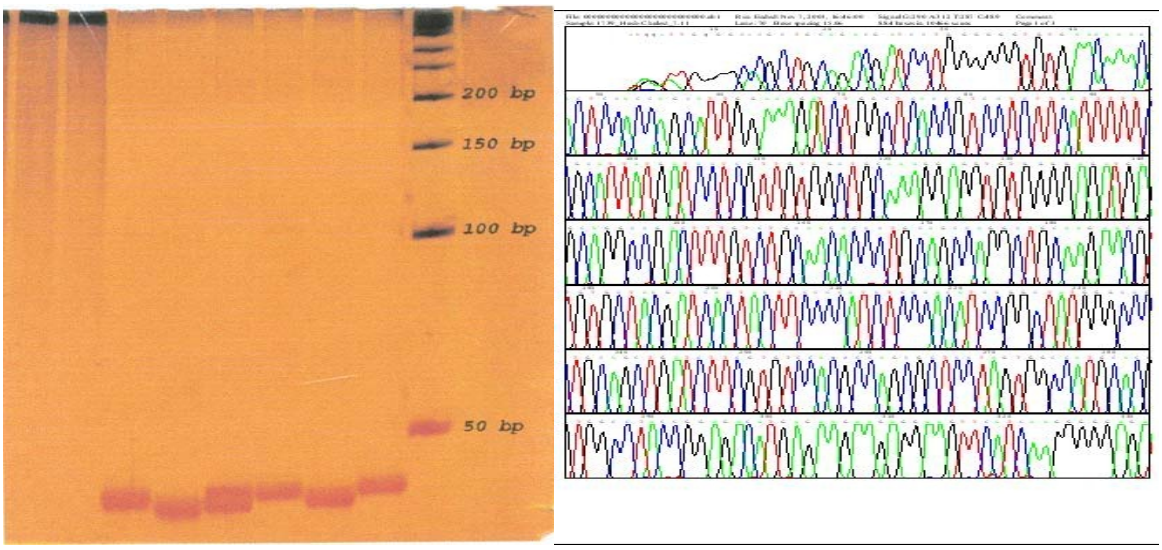

Fig.2: Sample of PAG electerophoresis & whole coding region sequencing

Results

Two handred and twenty four ARNSHL subjects from 189 families in two provinces of Iran (sistan va Baluchestan and Hormozgan) were investigated. Only one homozygote 35delG mutation and one heterozygote 35delG mutation were found in 189 families studied. The detected 35delG mutations were confirmed by sequencing. To investigate the other mutations in Cx26 gene, sequencing of the whole coding region of the gene was performed and a total of four Cx26 mutations including 35delG, W24X, R127H, and V27I+E114G were identified in 11 of 189 families (5.8%).Three polymorphisms including V27I, V153I and S86T also were found (Table 1). We identified 4 of 189 families (2.1%) with homozygous and compound heterozygous for Cx26 mutations.

Table 1: Cx26 gene variants identified in 189 ARNSHL families

Genotype	Sistan va Bluchestan Families No.	Hormozgan Families No.	Total
35delG/35delG	0	1(0.95%)	1(0.52%)
35delG/wt	0	1(0.95%)	1(0.52%)
W24X/W24X	1(1.1%)	1(0.95%)	2(1.05%)
V27I/E114G	1(1.1%)	0	1(0.52%)
V27I/V27I	0	1(0.95%)	1(0.52%)
V27I/wt	0	4(3.8%)	4(2.1%)
R127H/wt	2(2.3%)	4(3.8%)	6(3.1%)
V153I/V153I	1(1.1%)	1(0.95%)	2(1.05%)
V153I/wt	6(7.4%)	1(0.95%)	7(3.6%)
S86T/S86T	84(100%)	105(100%)	189(100%)

Discussion

A nested PCR strategy was used to investigate 35delG mutation alleles in 15% polyacrylamid gel. This is a very simple and reliable method for prescreening of 35delG mutation (6).

Eighty four deaf families from sistan va Baluchestan province (border line province near Pakistan) were studied. Three Cx26 mutations, W24X, R127H, V27I+E114G and two different polymorphisms, V153 I and S86T were identified. Surprisingly, no 35delG mutation was found in the families studied.

One hundred and five deaf families were also studied in Hormozgan province in south of country.Three Cx26 mutations, 35delG, W24X, R127H and 3 polymorphisms, V153I, V27I and S86T were identified. The common mutation (35delG) was found in 2 families (1.05%) from which only one family was 35delG homozygous. Totally 7 different genetic variants were identified in 25 of 189 families (13.2%) from which the Cx26 mutations were only found in 5.8% of them. Such low rate of Cx26 mutation is agree with the data have been reported previously in some British Asian, Omani, Japanese and Palestinian (7, 8). While a high rate of allele frequency for the 35delG (26%) has been reported before in Gillan province of Iran (9), no allele frequency was found for 35delG in Sistan va Baluchestan province. In fact this is the lowest rate of 35delG mutations reported from Iran so far (10). This low rate of 35delG in these two provinces are not comparable even with other provinces of Iran, that have shown in Fig. 3 (9).

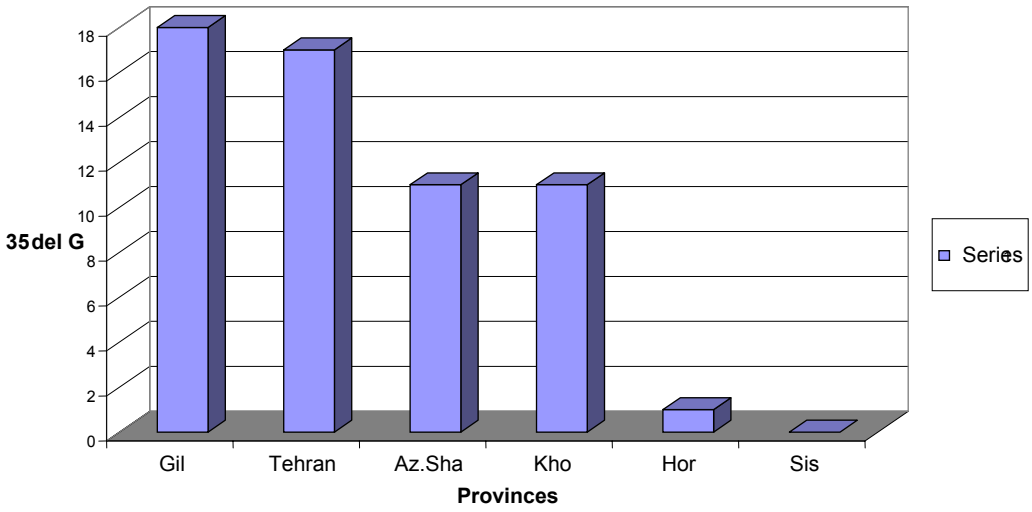

Fig. 3: Comparison of 35 delG in 6 Provinces of Iran

These data representing a huge variations regarding the prevalence of GJB2 mutations among different ethnic groups and populations. The lower rate of GJB2 diversity in these two provinces is presented in Fig. 3. While some populations are isolated, the others are impressed by the neighboring ethnic groups. The isolation of the populations can affect the mutation spectra of the GJB2 gene.

It is indicated that the contribution of the GJB2 gene mutations in ARNSHL in Sistan va Baluchestan and Hormozgan provinces are too low. Therefore, we would expect the contributions of other genes causing deafness in these provinces.

The result of this study should not generalized to Iranian people because Bluches are only 2% of whole Iranian population.

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References

1. Baris I, Kilinc MO, Tolun A (2001). Frequency of the 35 delG mutation in the connexin 26 gene in Turkish hearing-impaired patients. *Clin Genet*, 60: 452-55.
2. Mueller RF, Nehammer A, Middleton A, Houseman M, Taylor GR, Bitner–Glindzciz, et al (1999). Congenital non–yndromal sesorineural hearing impairment due to connexin 26 gene mutations -molecular and audiological findings. *Int J Ped Otorhinolaryngolo,* 50: 3-13.
3. Rickard S, Kelsell DP, Sirimana T, Rajput K, MacArdle B, Bitner–Glindzciz M (2001). Recurrent mutations in the deafness gene GJB2 (connexin 26) in British Asian families. *J Med Genet*, 38:530-33.

4. White TW (2000). Functional analysis of human CX26 mutations associated with deafness. *Brain Research Reviews*, 32: 181-83.
5. Murgia A, Orzan E, Polli R, Martella M, Vinanzi C, Leonardi E, Arslan E, Zacchello F (1999).CX26 deafness: mutation analysis and clinical variability. *J Med Genet*, 39: 829-32.
6. Hashemzadeh Chaleshtori M, Farhud DD, Taylor R, Hadavi V, Patton MA , Afzal AR (2002). Deafness-Associated Connexin 26 Gene (GJB2) Mutations in Iranian Population. *Iranian J Publ Health*, 31: 75-9.
7. Saroko Abe, Shin–ichi Usami, Hideichi Shinkawa, Philip M Kelley, William J Kimberling (2000). Prevalent connexin 26 gene (GJB2) mutations in Japanese. *J Med Genet*, 37: 41-43.
8. Brown KA, Janjua AH, Karbani G, Parry G, Noble A, Crock ford G, Bishop DT et al (1996). Linkage studies of non–syndromic recessive deafness (NSRD) in a family originating from the Mirpur region of Pakistan maps DFNBI centromeric to DI 13S175. *Human Mol Genet*, 5: 169-73.
9. M Hashemzadeh Chaleshtori, Doulati (2004). Two novel mutations and predominant 35delG mutations in the connexin 26 gene (GJB2) in Iranian population. *Iranian J Publ Health*, 33(2): 14-9.
10. Najmabadi H, Cucci RA , Sahebjam S, Kouchakian N, Farhadi M, Kahrizi K, Arzhangi S et al (2002). GJB2 Mutations in Iranians with Autosomal Recessive Non-syndromic Sensorineural Hearing Loss. *Human Mut*, Mutation in Brief#504 Online.